

Genetic polymorphisms of CYP2D6 oxidation in patients with systemic lupus erythematosus

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Abstract

Introduction: Systemic lupus erythematosus (SLE) is a complex, multifactor autoimmune disease. The studies on aetiopathogenesis of autoimmune diseases focus on the impact the genetically conditioned impairment of xenobiotic metabolism may exert. The knowledge of oxidation polymorphism in the course of SLE may be helpful in choosing more efficient and safer therapy. We determined whether there was an association between susceptibility to SLE and particularly to *CYP2D6* genotypes.

Material and methods: The study was carried out in 60 patients with SLE and 129 healthy volunteers and all the subjects were of Polish origin. The samples were analysed for two major defective alleles for *CYP2D6* – *CYP2D6*3* and *CYP2D6*4* and one wild -type allele *CYP2D6*1*-by the polymerase chain reaction fragment length polymorphism (PCR-RFLP) metod with DNA extracted from peripheral blood.

Results: No statistically significant differences in the incidence of *CYP2D6* genotypes between the studied groups were found ($p = 0.615$). Risk (OR) of SLE development was 1.03 for the carriers of *CYP2D6*3* allele and 1.48 for the subjects with *CYP2D6*4* allele; but it was not statistically significant.

Conclusions: Increased occurrence of mutant alleles of the *CYP2D6* gene in SLE patients and the calculated OR values could suggest the effect of these mutations on increased SLE development.

Key words: genetic polymorphism, oxidation, systemic lupus erythematosus.

Introduction

In recent years, numerous observations have elucidated the role of hereditary factors in susceptibility to some diseases. Systemic lupus erythematosus (SLE) is one of them.

Systemic lupus erythematosus is a chronic, systemic autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors. Systemic lupus erythematosus predominantly affects women in childbearing age. The disease is characterized by autoantibody production, abnormalities in the immune-inflammatory system function and inflammatory manifestation in several organs [1]. The aetiology of SLE is still unclear. The pathogenic process involves a complex interaction between environmental agents, hormones, immunomodulatory factors and disease susceptibility genes that predispose to SLE. The disease becomes symptomatic when the total of effects of different factors exceeds

a certain threshold, which causes an improper production of autoantibodies [2]. The genetic background of SLE is thought to be complex and involves multiple genes encoding different molecules with significant functions in immune system regulation [1, 3-5]. The group of candidate genes includes those encoding the activity of enzymes that metabolise xenobiotics. Environmental factors have been known to play a crucial role in autoimmune diseases; therefore numerous studies are being performed whose aim is to elucidate the effect of xenobiotics on the occurrence of autoimmune processes leading to SLE development. Many xenobiotics may induce alterations in the chromosomal structure, e.g., drugs, pesticides, fungicides, or substances causing environmental pollution [6]. The studies on aetio-pathogenesis of autoimmune diseases focus on the impact the genetically conditioned impairment of xenobiotic metabolism may exert.

Many of these enzymes, including CYP2D6, play an instrumental role in the oxidation of multiple drugs and in the activation and deactivation of environmental xenobiotics such as carcinogens and toxic substances [6-8]. The *CYP2D6* gene is localized on chromosome 22q13.1 [9]. The CYP2D6 (also called debrisoquine/sparteine hydroxylase) polymorphism was described in the 1970s while studying the metabolism of debrisoquine and sparteine [10]. Two phenotypically distinct groups were distinguished based on the capability to oxidise debrisoquine or sparteine to hydroxy-metabolites in the human population: persons actively oxidizing the above-mentioned compounds – extensive metabolisers (EM) – and subjects who oxidize poorly, to a minimum degree – poor metabolisers (PM). In general, PM patients are characterized by reduced or no metabolic activity of CYP2D6 enzyme compared to EM patients, who have normal metabolic activity of CYP2D6 [11]. The polymorphism of the *CYP2D6* gene has been relatively well explained, and more than 90 allelic variants of the *CYP2D6* gene have been described. (<http://www.imm.ki.se/CYPalleles>). These variants result from point mutations, deletions or additions, gene rearrangements and deletion or duplication of the entire gene and result in an increase, reduction, or a complete loss of activity [6]. In general, 71% of *CYP2D6* alleles in Caucasians are functional alleles, while non-functional alleles account for 26%. The non-functional *CYP2D6*4* (allele frequency 20%) is carried by 75% of CYP2D6 poor metabolizers, while *CYP2D6*3* is carried by 1-2% of them [12, 13]. The polymorphisms of the *CYP2D6* gene can result in defective or increased enzyme activity and *CYP2D6* genotypes usually exhibit large inter-ethnic differences [8]. The knowledge of oxidation polymorphism in the course

of SLE may be helpful in choosing more efficient and safer therapy, particularly in the case of a disease involving various organs and treated with drugs belonging to diverse therapeutic groups.

Studying gene-environment interactions in relation to the risk of SLE may be valuable because positive findings would clearly implicate the disease-causing exposures, clarify SLE aetiology and point to environmental modifications for disease prevention.

Despite the important role that CYP2D6 plays in detoxifying xenobiotics, few studies have investigated its function in SLE pathology [14, 15]. Our study is the first that estimates the distribution of *CYP2D6* genotypes in patients suffering from systemic lupus erythematosus from Poland.

The aim of our study was to analyse the *CYP2D6* alleles, to assess the distribution of the genotypes in 60 patients with SLE and to establish whether there is any correlation between *CYP2D6* genotype and SLE development.

Material and methods

The study was carried out in 60 patients with SLE diagnosed according to the criteria of the American College of Rheumatology [16]. The group of patients with SLE consisted of 54 women and 6 men (age range 17-76 years, mean 42 ± 12.48 years). The patients were hospitalized at the Department of Dermatology and Paediatric Dermatology, Military-Medical Faculty, Medical University of Lodz, Poland, and all the subjects were of Polish origin. The control group consisted of 129 volunteers (80 women and 49 men) aged 18-73 years (mean age 41.3 ± 15.41 years) with no acute or chronic autoimmune diseases. The local ethics committee on human research approved the study, and informed consent was obtained from all patients.

The samples were analysed for two major defective alleles for *CYP2D6* – *CYP2D6*3* and *CYP2D6*4* – and one wild-type allele *CYP2D6*1* by the polymerase chain reaction fragment length polymorphism (PCR-RFLP) method according to the procedure proposed by Smith *et al.* [17]. The application of the PCR-RFLP method allowed us to isolate two phenotypically distinct oxidation groups of subjects, i.e. poor metabolizers (PM), homozygotes possessing two mutated alleles: *CYP2D6*3* and *CYP2D6*4*; extensive metabolizers (EM), heterozygotes possessing one wild-type allele (*CYP2D6*1*) and one mutated allele (*CYP2D6*3*, *CYP2D6*4*); and homozygotes possessing two wild alleles (*CYP2D6*1*) in their loci.

Genomic DNA was isolated from peripheral blood leukocytes. A 10 ml venous blood sample was obtained from each patient and control and was stored in EDTA blood containers at -20°C . Genomic

Table I. Distribution of poor and extensive genotypes in patients with SLE and in the controls

Group	Number of patients	Poor metabolizers (PM)	Extensive metabolizers (EM)
SLE	60	7 (11.7%)	53 (88.3%)
Control	129	12 (9.3%)	117 (90.7%)

$\chi^2 = 0.253$, $p = 0.615$, p – statistical significance ($p < 0.05$), χ^2 – test χ^2 comparison between 2 groups of patients with SLE and control subjects

DNA was subjected to amplification using PCR with a pair of synthetic oligonucleotides, separately for the *CYP2D6*3* and *CYP2D6*4* mutations. PCR amplification was performed in a total reaction volume of 50 μ l containing 0.2–0.5 μ g genomic DNA, 1U Taq Polymerase (Invitrogen USA), 1 \times PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.5 mM MgCl₂, 0.2 mM of each dNTP (Fermentas, Germany), 5% DMSO (Sigma, USA) and 250 nmol of each primer (Oligo IBB PAN, Poland). PCR amplification was conducted in an Eppendorf *Mastacycler Personal* thermal cycler (Germany). The obtained product of amplification was subjected to digestion by restriction enzymes after control assessment in 2% agarose gel. The restriction endonucleases BstN 1 (New England BioLabs, USA) (*CYP2D6*4*) and HpaII (Invitrogen USA) (*CYP2D6*3*) were used to detect the presence or absence of mutations at the position G 1934 A and A 2637 deletion, respectively. Separation of the obtained digestion products was conducted using electrophoresis in 8% polyacrylamide gel. The obtained bands corresponding to particular DNA fragments following staining of the polyacrylamide gel with ethidium bromide were analysed in UV light.

The frequency distribution of *CYP2D6* genotypes in SLE patients was compared with healthy subjects and analysed statistically using the χ^2 test alone and with the Yates modification for small groups. Odds ratio (OR) with 95% confidence interval

(95% CI) was calculated using the computer program Statistica 6.0.

Results

We determined whether there were any associations between susceptibility to SLE and particular *CYP2D6* genotypes. Subjects were assigned to three different classes of overall genotypes (PM: *CYP2D6*3/CYP2D6*4* or *CYP2D6*4/CYP2D6*4*, heterozygous EM: *CYP2D6*1/CYP2D6*4* and homozygous EM: *CYP2D6*1/CYP2D6*1*) on the basis of PCR results.

The frequency distribution of oxidation genotypes determining poor and extensive metabolizers in patients suffering from systemic lupus erythematosus and in controls is shown in Table I. There was no significant difference in the frequency of distribution of poor and extensive genotypes between patients with SLE and the control group.

The incidences of the three possible genotypes in the SLE population and in the controls as well as the OR SLE development are summarized in Table II. Among 60 patients with SLE, EM homozygotes of the *CYP2D6*1* allele with *CYP2D6*1/CYP2D6*1* genotype accounted for 45%, and heterozygotes with *CYP2D6*1/CYP2D6*4* genotype accounted for 41.67%. Seven (11.67%) people were PM with *CYP2D6*3/CYP2D6*4* or *CYP2D6*4/CYP2D6*4* genotype. Results obtained in the SLE group did not differ significantly from those in the control group.

Frequency of *CYP2D6* gene alleles was compared between SLE patients and the control group. Differences in allele frequency between the groups were not statistically significant (Table III).

Discussion

The human organism is constantly exposed to harmful exogenous factors (xenobiotics) including drugs and carcinogenic compounds that can induce development of a large number of diseases. The

Table II. Frequency distribution of homozygous EM and PM and heterozygous EM patients with SLE and controls

CYP2D6	Patients with SLE ($n = 60$)		Control group ($n = 129$)		Value of p	OR (95% CI)
	N	%	N	%		
Extensive metabolizers (EM)	Homozygous:					
	<i>CYP2D6*1/CYP2D6*1</i>	27	45	74	57.4	1.112
	Heterozygous:					
	<i>CYP2D6*1/CYP2D6*3</i>	1	1.66	4	3.1	0.571
Poor metabolizers (PM)	<i>CYP2D6*1/CYP2D6*4</i>	25	41.67	39	30.2	1.122
	Total:	53	88.33	117	90.7	0.615
	<i>CYP2D6*3/CYP2D6*4</i>	1	1.67	0	–	0.141
	<i>CYP2D6*4/CYP2D6*4</i>	6	10	12	9.3	0.879
Total:		7	11.67	12	9.3	0.615
						1.29 (0.48-3.46)

processes of biotransformation in the organism are multidirectional and xenobiotics can be transformed into active or inactive metabolites via the oxidative route. They can also be transformed into harmful compounds with a potentially pathogenic action. The enzymatic system associated with the liver microsomal fraction with cytochrome P-450 as its fundamental element is responsible for xenobiotic oxidation. At present, the role of cytochrome P-450 and its isoforms in regulating not only the processes of drug metabolism and immune reactions but also in activating toxic substances has aroused great attention in the literature. Cytochrome P-450 enzymes show genetic polymorphism, which contributes to marked inter-individual differences in drug responses. Examinations of the oxidative genotype or phenotype have been performed in order to identify the oxidative polymorphisms [18, 19]. Differences among individuals in drug responses due to mutations in the *CYP2D6* gene that is involved in the metabolism of xenobiotics, including a number of drugs with a wide therapeutic use, were discovered as early as in the 1970s. Genetically determined oxidative polymorphism can affect not only drug efficacy but may also be a factor predisposing to disease development.

In the present study we tried to answer the question whether any associations exist between genetically determined activity of CYP2D6 enzyme and SLE development in the Polish population. There are numerous reports demonstrating the role of oxidative polymorphism in predisposing towards development of tumour diseases, allergy or psychiatric and neurological syndromes. Studies evaluating a predisposition towards tumour diseases have been carried out for a long time and predominantly concern tumours of the lungs, genitourinary tract and prostate as well as myeloblastic leukaemia. Those studies demonstrate that subjects who are extensive or ultra-rapid metabolizers account for the majority of patients with tumour diseases [20-24]. Studies on oxidative polymorphism have also been conducted in patients with Parkinson's or Alzheimer's diseases; however, the results are conflicting [13, 25, 26].

There are few reports in the available literature that concern the role of xenobiotic metabolizing enzyme polymorphism in autoimmune diseases [14, 15, 27]. However, a report about the association of *CYP2D6* gene with SLE is still unavailable in Poland. Systemic lupus erythematosus is a model autoimmune disease that is characterized by increased production of autoantibodies against different antigens. Contemporary medicine is able to alleviate the symptoms only to a limited extent, with no possibility of complete recovery. The reason for that is still insufficient knowledge on SLE

Table III. Frequency of CYP2D6 alleles in patients with SLE and controls

Allele	<i>CYP2D6*1</i>	<i>CYP2D6*3</i>	<i>CYP2D6*4</i>
Patients with SLE (%)	80 (66.6%)	2 (1.7%)	38 (31.6%)
Controls (%)	191 (74%)	4 (1.6%)	63 (24.4%)
OR (95% CI)	0.7 (0.44-1.11)	1.08 (0.19-5.96)	1.43 (0.89-2.29)

aetiopathogenesis, but there is evidence for a crucial role of genetic factors besides immunomodulatory and environmental factors in the disease development.

The first reports concerning associations between oxidative genotype and SLE were published in 1998. A study that was conducted among 69 patients with SLE and 514 healthy Caucasian volunteers showed that the incidence of the *CYP2D6*4* allele was significantly higher in the group of SLE patients compared with the control group (28.3% and 18.6%, respectively). However, the difference was not statistically significant. It has been observed that the *CYP2D6*4* allele occurred more frequently in SLE patients in whom renal involvement and haematological abnormalities had been established [15]. *CYP2D6* polymorphism was also the subject of a study by Kortunay *et al.* However, the authors failed to show any association between the *CYP2D6* genotype and the risk of developing SLE [14].

In our own studies we observed a higher proportion of subjects who were characterized by poor metabolism among SLE patients in comparison with the healthy ones. It has been shown that the frequency of genotypes for the poor and extensive oxidation in the group of SLE patients reached 11.7% and 88.3% respectively compared with 9.3% for poor metabolizers and 90.7% for extensive metabolizers in the control group. No statistically significant differences in the incidence of *CYP2D6* genotypes for poor and extensive oxidation between the studied groups were found ($p = 0.615$). *CYP2D6*1/CYP2D6*1* and *CYP2D6*1/CYP2D6*4* genotypes occurred most frequently in the group of SLE patients, whereas *CYP2D6*1/CYP2D6*1* genotype was predominant among the controls. Relative risk of developing SLE, expressed as the odds ratio (OR), was not statistically significantly higher for the carriers of the particular genotypes.

The wild-type allele *CYP2D6*1* was found most frequently (66.6%) in the group of patients with SLE. Both mutant alleles, i.e. *CYP2D6*3* and *CYP2D6*4*, accounted for 1.7% and 31.6%, respectively, and these values were not statistically

significantly different from the results obtained both in the control group (74%, 1.6% and 24.4% respectively) and the results in the Caucasian population (70%, 2% and 20% respectively) [23, 28, 29]. Relative risk of SLE development was 1.08-fold higher for the carriers of the *CYP2D6*3* allele and 1.43-fold higher for the subjects with the *CYP2D6*4* allele; however, the risk was not statistically significantly higher. Increased occurrence of mutant alleles of the *CYP2D6* gene in SLE patients and the calculated OR values could suggest the effect of these mutations on increased SLE development. Continuing the studies in a larger group of SLE patients is likely to provide confirmation of the observations and a more precise insight into the nature of the relationships between genetically determined metabolic factors and SLE development.

There are reports in the literature on the impact of the polymorphisms of other cytochrome P-450 enzymes on the risk of SLE development. Results obtained from the studies on the *CYP1A1* gene implicate that the presence of polymorphisms in this gene might be a risk factor for SLE development and a predictor of renal involvement [30].

Knowledge of the genetically conditioned differences in oxidation processes as well as determination of the incidences of the principal mutations in the cytochrome P-450 isoenzyme *CYP2D6* gene seems to be a vital clinical issue. Pharmacogenetic studies provide the possibility of establishing one of the potential reasons for the risk of developing autoimmune diseases, including SLE. Results from the studies that have been conducted so far allow the statement that oxidative polymorphisms are not a risk factor for developing SLE.

However, it seems that determination of *CYP2D6* genotype in SLE patients is of significant clinical value since SLE is a disease that involves many organs and the treatment may require application of drugs that are subject to oxidation. Therapeutic effects with drugs subjected to oxidation are less satisfactory in extensive metabolizers compared with persons who are poor metabolizers. Relapses occur more frequently and remissions are quite rare among subjects who are extensive metabolizers. Treatment seems more effective in poor metabolizers but serious side effects that are associated with applying standard doses of drugs are frequently observed.

An examination of the oxidative genotype or phenotype allows individualization of pharmacotherapy. Knowledge of the oxidative polymorphism and its relationship with SLE may contribute to a safer and more efficient therapy since the disease is multiorgan and often requires drugs that belong to a variety of therapeutic groups.

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